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(71) Applicant *(for all designated States except US)*: MICROCROP (IRELAND) LIMITED [IE/IE]; St. Stephen's Green House, Dublin 2 (IE).

(71)(72) Applicant and Inventor: PERRIN, Alan, Phillip [GB/IE]; The Masters, Castledermot, Kildare (IE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : CASSELLS, Alan [GB/IE]; Bridge House, Guileen, North Whitegate, Cork (IE). O'SHEA, Anthony [IE/IE]; Braunshill, Carlow (IE). LONG, Roger [IE/IE]; Aghold Rectory, Coolkenno, Tullow, Carlow (IE). HURLEY, Deirdre [IE/IE]; 5 Tyndall Court, Kilkenny Road, Carlow (IE).

(74) Agent: GEOFFREY OWEN & COMPANY; 76 Lower Bridge Street, Chester CH1 1RU (GB).

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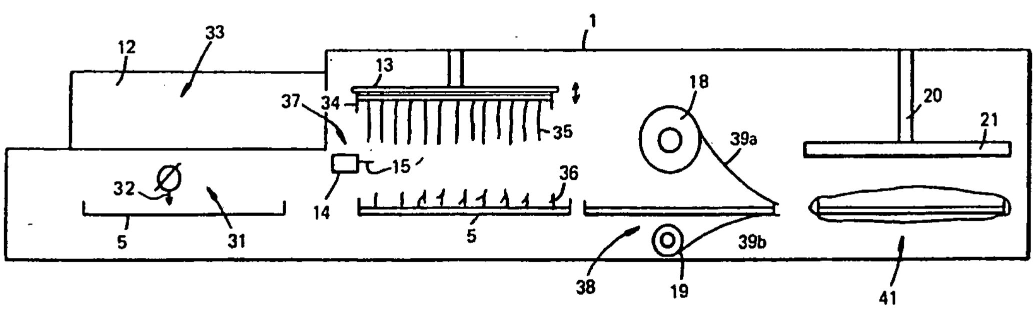

(57) Abstract

The invention provides apparatus for severing plant material in micropropagation. Microplants (35) growing in a culture-medium-containing tray (34) are supported in an inverted disposition within an enclosure (1). A cutting means (15) suitably having one or more optionally cooperating blades is traversed across the array of microplants (35) at a level such that a multiplicity of nodes (36) are cut from the inverted plants (35). The nodes (36) are collected on an underlying container or tray (5), containing a culture medium. The cut nodes held on tray (5) are then enclosed within a PVC wrapping material (39a, 39b) at a wrapping station (38), sealed within the film (39a, 39b) at a further station (41), and removed to a downstream growth location, where plant development takes place under controlled environmental conditions.

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"Improved micropropagation apparatus and method"

This invention relates to the cutting of growing plant material. The present invention also relates to apparatus and methods for the micropropagation of plants. In particular, the invention relates to the cutting of growing plant material in the micropropagation of plants. The invention is especially directed to cutting apparatus for use in plant micropropagation.

At its simplest, micropropagation consists of excising small pieces of actively growing tissue, and, under sterile conditions, transferring them to a nutrient growing medium in test tubes or other suitable containers. These cultures may then be grown on to produce microplants. The excised shoot tips or meristems of many plant species may be cultured on a relatively simple medium. This has proved a valuable method of obtaining true to type plants from organised bud tissue which is also virus free. This arises partly from the fact that the apices of many virus infected plants remain free of infection. Alternatively, the initial cutting may be taken from the leaf or internodal areas giving rise to the formation of

adventitious shoots. The use of callus cultures and adventitious shoots may however result in a loss of the morphogenetic potential of the tissue or an increase in genetic variability.

Nodal cuttings of microplants are thus suitably taken under sterile conditions; nodal cutting involves cutting the stem of the plant between nodes to ensure that the cuttings contain an axillary meristem. Typically, approximately ten nodal cuttings may be placed in a sterile container containing nutrient salts, a carbon source, and agar, with each cutting end inserted into the medium. The cuttings are then grown in a growth room under specified temperature and lighting conditions, and each bud grows to produce a shoot which may again be cut nodally to increase the stock of plants. Once the stock is increased sufficiently the cuttings may be grown on and rooted to produce the final plants.

Two important issues in plant micropropagation are the origin of the shoots and the efficiency of production. In order for plant material such as the potato to be produced true to type from tissue culture, the shoots must arise from preformed, organised bud tissues, or a phenomenon known as somaclonal variation can occur. With some ornamental crops however, this is not a problem on account of the stability of the particular genotypes involved and also because variation within the crop is not necessarily disadvantageous. However, for potatoes, two criteria for obtaining certification of seed potatoes are freedom from disease and trueness to type. Trueness to type may suitably be achieved by ensuring that the microplants always arise from distinct axillary buds.

The second problem in commercial plant micropropagation adverted to above is the efficiency of production of new plants. Several methods are available for _in vitro_ propagation of plants, in particular the potato. The technique developed by Hussey and Stacey (Ann. Bot. _48_, 787-796 (1981) and Ann. Bot. _53_, 565-578 (1984) is based on the proliferation of axillary buds. A further problem in commercial

micropropagation relates to the presence of adventitious regeneration from somatic cells.

Conventional micropropagation processes are both highly skilled and labour intensive and are therefore both costly and slow. Various methods have been suggested by which the known manual process may be mechanised. According to European Patent Application No. 84305068.3 of Milouda Ltd., a blender is used to chop the microplants to produce nodal cuttings. However, this system does not appear to have been used commercially and the origin of the shoots from the macerated tissue pieces may be doubtful.

An alternative has been suggested in PCT Publication No. 0088/02231 (Application No. PCT/HU86/0053) of Novotrade RT. The process described in this patent specification is satisfactory, but does not improve on recognised, state of the art, manual micropropagation.

It is an object of the present invention to provide improved apparatus and methods for micropropagation directed to overcoming at least some of the disadvantages recited above of the known manual method and of previous attempts at mechanisation.

According to the present invention, in a first aspect, there is provided apparatus for severing plant material in micropropagation comprising:

(a) cutting means, and

(b) means for holding a multiplicity of microplants for a cutting operation, wherein the disposition of the holding means during a cutting operation is such that plant material severed from said multiplicity of microplants during said cutting operation falls free of said multiplicity of microplants.

In a particular arrangement, there is provided an apparatus for the mechanisation of plant micropropagation comprising a chamber which can be maintained under sterile conditions and which houses a

holding means to hold a culture container containing microplants and a cutting means to cut the microplants into nodal cuttings, the holding means holding the container in a position such that the nodal cuttings can fall free of the microplants and container once cut.

Preferably the holding means holds the container in an inverted position with respect to the normal growing position such that the microplants are suspended from the culture container above the cutting means.

The apparatus may also comprise a means to invert the culture container.

Preferably the cutting means comprises at least one pair of inter-digitated blades which can be moved along the inverted microplants. Preferably the means to hold the culture container in the apparatus comprises at least one runner with which the culture container is engageable. Alternatively, the holding means may comprise one or more magnets, the culture container being made of a magnetic material, such as metal, so that the culture container may be held in position by the magnet or magnets.

The means to hold the culture container may suitably be raised or lowered with respect to the cutting means so that successive cuts may be taken from the microplants. Alternatively, the cutting means may be movable relative to the holding means. In a preferred embodiment, the apparatus comprises an initial medium-filling station, at which a culture container may be filed with culture medium from a medium-containing reservoir, and a means to move the container into a location beneath the cutting head such that as the inverted microplants are cut, the nodal cuttings fall into the container beneath.

The medium reservoir may be provided with a pump to pump medium into the container.

Preferably the apparatus also comprises a means for aseptically sealing the container containing the newly-produced microculture. The sealing mechanism may comprise one or more rolls of PVC film which can be fed around the culture container and then heat-sealed in a conventional manner around it.

Preferably the apparatus is provided with means to introduce filtered air under positive pressure into the chamber, in order to maintain the sterility of the system. The apparatus may also comprise a sterile entrance chamber through which the microplants to be nodally cut can be aseptically introduced into the apparatus.

Preferably the entire apparatus is microprocessor-controlled.

The invention also extends to microculture containers for use with the above-described apparatus. The containers may comprise a microculture tray which is engageable with an outer casing which in turn is engageable with the container-holding means of the micropropagation apparatus. Alternatively the microculture tray may itself be engageable with the container-inverting means of the apparatus.

The container may comprise a groove along each of at least two of its sides which is engageable with a ridge on the container-holding means. Alternatively the holding arrangement may comprise a T-shaped groove on the base of the container which is engageable with a runner on the holding means or vice versa.

In a further variant, the container may be made of a magnetic material which will engage with a magnet in the container-holding means, or vice versa.

The container is however preferably elongate and made of extrüded PVC.

In any of the foregoing embodiments of the invention, the cutting

means may comprise at least one single-element cutter as subsequently defined hereinafter.

In a second aspect, the invention is directed to a method of severing plant material in micropropagation comprising holding a multiplicity of microplants for a cutting operation in a disposition such that plant material severed from said multiplicity of microplants during said cutting operation falls free of said multiplicity of microplants.

In a particular realisation of this aspect of the invention, there is provided a mechanised process for the micropropagation of plants in which microplants, in a sterile environment, are inverted over a mechanical cutting means which cuts nodes from the microplants, the nodes being collected onto a culture-medium-containing tray held beneath the cutting means, and the tray containing the cut nodes is then aseptically sealed and cultured to produce microplants.

The invention also relates to plants or microplants produced by the apparatus or the process of the invention.

The specific cutting means described above includes at least one pair of inter-digitated blades, i.e. blades co-operating in the manner of a sheep-shearing head or barber's trimming apparatus to slide laterally relative to one another, so that the material to be cut is trapped and severed in the manner of a multiplicity of simultaneous scissors-type cutting operations.

A particular problem arising with cutters of this kind is contamination. Plant material tends to accumulate on the blade structure, and its removal and sterilisation of the cutter presents considerable problems. Furthermore, in micropropagation, the plants tend to be relatively weak and also long in relation to their other dimensions. In these circumstances, use of an inter-digitating blade type cutter may result in the nodes tending to become stripped rather than a clean severance of the plant stem. These latter factors can result in loss of up to twenty-five percent of nodes.

It is therefore a further object of the invention to provide an improved cutter structure for use in micropropagation in particular, which will obviate at least certain of the disadvantages of earlier arrangements, as identified above.

According to the invention in a third aspect, there is provided therefore apparatus for severing plant material in micropropagation comprising:

(a) A cutting head having at least one single-element cutter,
(b) Means for holding a multiplicity of microplants for a cutting operation,
(c) Means for effecting displacement of said cutting head relative to said microplants, and
(d) Means for establishing a cutting relationship between said single-element cutter and said microplants.

By a single-element cutter is meant a cutter in which the cutting action takes place by virtue of engagement of a single cutting surface only with the plant material. A single-element cutter is to be distinguished from in particular a scissors-type cutter or other inter-digitating blade structures, in which the cutting action takes place by relative sliding engagement and overlapping co-operation of a pair of cutter blades.

In a favoured embodiment, said single-element cutter comprises a circular blade driven in rotation by said means for establishing a cutting relationship, so that a peripheral cutting edge of the blade severs the plant material. Cutting co-operation between the cutting head and the microplants is thus established by relative displacement between said cutting head and the microplants during rotational drive of the rotary blade.

Suitably the cutting head is mounted on a traversing carriage for drive relative to a container holding the microplants in an inverted

disposition. This transverse or traversing drive of the cutting head along or across suitably a longitudinal dimension of the container may be effected by a drive shaft or lead-screw type mechanism. Rotation of the circular blade may also be effected by a suitable drive mechanism set in motion by a drive shaft or lead-screw mechanism, which may be activated by the same drive shaft or lead-screw as that used for effecting the traversing drive. Alternatively and preferably, separate drives are provided for carriage or cutting head traverse and blade rotary motion. Said means for establishing a cutting relationship is thus defined in this embodiment of the invention by a blade drive feature, such as a motor or gear train.

Suitably, the apparatus comprises means for constraining the microplants against significant displacement in at least one direction relative to the direction of cutting head travel. In a preferred arrangement, constraint is also applied to prevent displacement of the microplants in a second direction at right-angles to said at least one direction, the microplants thus being then substantially constrained against displacement in two directions at right-angles to one another.

In an alternative embodiment, other types of single-element cutter may be employed. In particular, a non-physical-contact cutter, such as a laser cutter, may be used.

In any embodiment of this aspect of the invention, the cutting head may also be provided with an integral drive mechanism, not requiring any mechanical transmission of power. A battery driven electric motor may for example be used, in particular, for blade rotational drive. An arrangement of this kind particularly facilitates sterilisation and cleansing of the structure, in that the entire carriage or cutting head may be removed from the apparatus, including the drive mechanism and its power source, for independent external sterilisation.

The arrangement according to the invention as described above provides a particularly effective cutting structure especially suited to micropropagation. The high rotational speed of the circular cutter in the favoured embodiment results in a self-cleaning function, so that there is effectively no substantive or significant contamination whatever of the blade. The cutting action provided by this structure is especially effective in securing a high yield from the severed plant material, with a relatively low level of loss. The manner of cutting using the single-element rotary blade, preferably running at a relatively high rotational speed, also minimises plant stem damage, so that virtually all of the cuttings are usable, while the original microplant remains capable of further vigorous growth.

The arrangement of the invention in the present aspect is especially suited to use in systems of the kind where the microplants are supported in an inverted disposition and the severed nodes fall away from the cutter, i.e. wherein the disposition of the holding means during a cutting operation is such that plant material severed from said multiplicity of microplants during said cutting operation falls free of said multiplicity of microplants. However, it may also be applied to constructions in which the plants are held in an upright or normal growing disposition. In this instance, a sheathing cover may be provided to conceal the upper surface of the blade, and the cut plant material is then collected on this sheathing surface.

The invention may also make use of two or more single-element cutters working in co-operation with each other. In a particularly favoured arrangement, a plurality of rotary circular blades is provided, mounted in a substantially planar array, with alternate blades running in contra-rotation.

The invention will now be described with reference to the accompanying drawings in which:-

Figure 1 is a schematic view of a method of axillary bud or nodal culture of the potato (for example),

Figure 2 is a flow diagram of automated nodal culture of potatoes, according to the invention,

Figure 3 is a diagrammatic representation of apparatus according to the invention,

Figure 4 is a side view of cutting and loading stations of micropropagation apparatus according to the present invention,

Figure 5 is a side view of a wrapping station of the apparatus of Figure 4,

Figure 6 is a view on the line A-A of Figure 5,

Figure 7 is a view on the line B-B of Figure 5,

Figure 8 is an end view on the line C-C of Figure 4 of the cutting and loading station of Figure 4, showing a container feed arrangement,

Figure 9 is a schematic pictorial representation of a further form of cutting apparatus according to the present invention,

Figure 10 is a schematic pictorial detail view of a microplant constraining member, as also shown in outline in Figure 9,

Figure 11 is a diagrammatic end view showing an array of microplants in position in the apparatus of Figure 9 preparatory to cutting, with constraining members of the kind shown in Figure 10 in position,

Figure 12 shows in diagrammatic representation an arrangement of cutting apparatus having a plurality of contra-rotating planar circular blades, and

Figure 13 shows in schematic side sectional view, an arrangement for collecting severed nodes from plants oriented in their normal growing direction during cutting.

The production of potato microplants is initiated from disease free meristem cultures by way of a procedure known as axillary bud culture or nodal culture, as depicted diagrammatically in Figure 1.

The meristem culture consists of a small rooted plant enclosed in a sterile jar having both leaves and buds where the leaf stalk joins the stem. (Steps 1 and 2). These plants are then subcultured under aseptic conditions (Step 3) by excising the buds and placing them on sterile containers containing a standard tissue culture medium of 2.35 g/l Murashige and Skoog salts (Physiologia Planetarium <u>15</u> pp473-97, (1962)), 15g/l sucrose and 6g/l agar. The pH of this medium is adjusted to 5.8. Cultures are placed in a growth room having a 16 hour photoperiod and a temperature of 20°C. The individual buds then grow into small plantlets, the entire process taking approximately four weeks (Step 4). This cycle can then be repeated, each plant regenerating about five more in a four week period.

A simplified flow chart of an automated process provided by the present invention as it applies to potatoes is presented in Figure 2.

Initiation of cultures for the automated process is similar to that already described for the conventional process, differing only in the size of the tissue culture container used. Nodes are now placed manually into rectangular trays (30 X 9.5cm) capable of holding up to 300 nodes. (Steps 1 and 2).

These trays are placed in plastics containers which are in turn sealed and left for approximately four weeks in a growth room. The trays are then unwrapped under sterile conditions, inverted and inserted into the micropropagation apparatus of the invention via a

sterile transition chamber (Step 3). A mechanical cutter is used to cut the inverted plants (Step 4), the cut nodes falling onto füll-sized trays (approx. 120 x 9.5 cm) containing standard nodal medium. These trays, containing up to 100 nodes, are then wrapped in PVC film and passed through a hot plate device which effectively seals them before their removal from the apparatus or machine (Step 5).

The filled trays then grow for four weeks in the growth room to produce microplants (Step 6). The operations from Step 3 are then repeated until a sufficient number of microplants, all having their origin from the original culture, has been generated.

The micropropagation apparatus of the invention is shown diagrammatically in Figure 3. It has an outer housing or enclosure 1 into which filtered air is pumped to maintain sterility. Within the housing 1 there are a number of workstations at which the various operations of the micropropagation process according to the invention are performed. The first of these is a medium-filling station 31 where the culture containers 5 which are to receive the cuttings are filled with medium 43 (Figure 8) at filling point 32. Above this station 31, there is a loading station 33 where a container 34 of microplants 35 which are to be processed into nodal cuttings 36 is loaded into the apparatus, in the inverted position, through a sterile transition chamber 12.

The medium-filled container 5 is then moved on rollers 4 (Figure 4) into a cutting station 37. The container 34 of microplants 35 is also moved into the cutting station 37, the container 34 being held in an inverted disposition above cutting blades 15 mounted in a cutting head 14 so that as the nodes 36 are cut, they fall under gravity into the medium-filled container 5 beneath the blades 15. Container 34 is held in this inverted disposition by a tray holder or holding means 13, to be described in more detail.

A container 5 which has received nodal cuttings 36 is then moved or advanced further to a wrapping station 38 where it is wrapped in PVC film 39a, 39b, and the wrapped container 5 is then further advanced to a heat-sealing station 41 where the PVC film 39a, 39b is sealed around the container by a heat-sealing device 20 carrying a hot plate 21. The PVC film layers 39a, 39b are provided from upper and lower rollers, 18, 19. The wrapped and sealed container 5 is then off-loaded from the apparatus at a downstream discharge station.

The apparatus of the invention is shown in more detail in Figures 4 to 8. Referring initially to Figure 4, which is directed to the filling station 31, the loading station 33 and the cutting station 37, a fan 2 pumps air, which has already passed through a HEPA filter 3, through further ultrafilters into the housing 1. A series of rollers 4 are provided in a lower region of the enclosure of housing 1 along which the microculture containers 5 are moved through the apparatus from the medium-filling station 31 through the cutting station 37 and on to the wrapping 38 and sealing 41 stations. The microculture containers 5 are suitably elongate rectangular trays of extruded PVC, preferably having a longitudinal groove 6 extending along each elongate side adjacent the base 42 of the tray.

Figure 8 is an end view of the filling 31 and cutting 37 stations of the apparatus. Referring to this drawing, before being loaded into the apparatus at filling station 31, the empty trays 5 intended to receive nodal cuttings are manually fed into a magazine 7 and passed through a sterilizing soak tank 8 on a conveyor belt 9. The trays 5 are then fed into the medium-filling station 31 of the micropropagation apparatus where they are filled at 32 with culture medium 43 from a medium tank 10 via a pipeline 11. The trays 5 are advanced through the filling station 31 and transferred into the enclosure 1 by means of a laterally-oriented further conveyor 44. The trays 5 are then fed, one at a time, along the rollers 4 into the cutting station 37.

Considering now the loading station 33, a tray 34 containing microplants to be cut is manually inserted into the apparatus by way of a sterile transition chamber 12. As shown, tray 34 is identical with trays 5, but this is not essential. The plant-accommodating trays 34 may be of different configuration and dimensions from the receiving trays 5, if appropriate or required. The tray 34 is first inverted and is then pushed into a tray holder 13 located at the cutting station 37 above the level of the cutter 15 in an operative or cutting disposition of the apparatus. The tray holder 13 has two ridges or projecting ribs along its length which engage within the grooves 6 on the sides of the microculture tray 34 to hold this tray in position within the apparatus at the cutting station, in the inverted disposition or orientation shown. The holder 13 suitably also moves on rollers into the cutting station 37 from an initial tray-receiving location at the loading station 33. Thus the roller-mounted holder 13 may be displaceable in the longitudinal direction of the apparatus between the loading 33 and cutting 37 stations.

At the cutting station, there is mounted an electrically-powered cutting head 14 which comprises a pair of interdigitated blades 15. The cutting head 14 is supported by a mounting structure 16 above the culture-medium-containing tray 5 and below the inverted tray 34 containing the microplants to be cut. The height of the tray 34 mounted on or supported by the tray holder 13 above the cutting plane of the head 14 is adjustable by means of a pneumatic cylinder 16a, so that the microplants can be moved closer to or further away from the head 14. The cutting head 14 is displaceable along the length of the inverted tray 34 to enable the cutting action to be effected. The travelling displacement of the cutting head 14 required to effect this cutting action is controlled by a pneumatic cylinder 17.

Once nodal cuttings have been taken or made, they fall into the medium-containing tray 5 beneath the blades 15, and this tray 5 is passed or advanced along the rollers 4 into the wrapping station

38. The wrapping station 38 has an upper roller 18 and a lower roller 19, each of which holds a respective roll 39a, 39b of PVC film. The lower roller 19 lies beneath the tray-carrying or support rollers 4, while the upper roller 18 lies above the rollers 4. The tray 5 can thus pass between the rollers 18 and 19. Accordingly, as tray 5 moves into the wrapping station 38, it is sandwiched between two layers 39a, 39b of PVC film.

The wrapped tray 5 is then further advanced, again such as on support rollers, into the sealing station 41 which contains a heat-sealing device 20 having a hot-plate 21 for heat-sealing the PVC film around the tray 5. Once sealed, the tray 5 is pushed at 22 through outwardly-hinged doors out of the apparatus and is transferred to a growth room, for example, manually.

To produce nodal cultures using apparatus of the invention, as described above, a tray 34 which contains a number of microplants is inverted and positioned in the tray holder 13 in the cutting station 37. Meanwhile, an empty tray 5 is loaded into the apparatus by way of station 31, where it is filled with culture medium 43. The tray 5 is then passed or advanced on rollers 4 into the cutting station 37.

The spacing between the inverted tray 34 and the cutting plane, i.e. the height of the inverted microplants above the cutting head 14, is adjusted by raising or lowering the inverted tray 34 held by holder 13 so that the cutting head 14 is spaced by approximately 5 to 15 mm from the tips of the inverted microplants which are hanging in the cutting station 37, to overlap the plant shoot and define a plant portion of 5 to 15 mm in length to be cut off as a nodal cutting. This adjustment of the cutting plane level is effected by means of cylinder 16a, which regulates and controls the height adjustment of the container holder 13. The cutting head 14 supported by mounting structure 16 is then moved longitudinally along the length of the inverted tray 34. The interdigitated blades 15 are driven in a cutting action, to thereby sever the nodal cuttings as the cutting

head moves. As they are cut, the nodes fall into the receiving tray 5 which contains medium and is located beneath the cutter 14. This tray 5 is then moved onwards on the rollers 4 into the wrapping station 38 and a fresh tray 5 containing culture medium takes its place in the cutting station 37 for a further nodal cutting operation as required. For a further cut from the same plants, the height of the cutting head 14 relative to the inverted microplants is readjusted so that a further run of nodes, again of 5 to 15 mm, can be cut from the same microplants. Each tray 34 of microplants may undergo up to five cutting operations. Alternatively however, and preferably, only a single cut may be taken from the microplants in tray 34 and the plants may then be allowed to regrow to provide a further set of nodal cuttings to be taken from the tips of the growth. Thus, the cutting step may comprise either a single nodal tip cutting operation or a multiple cutting operation.

When a tray 5 has received freshly cut nodes, it is passed into the wrapping station 38 to be wrapped in PVC film which is then sealed around the tray 5. The tray 5 is then discharged from the apparatus of the invention and suitably transferred to a growth room where it remains until the nodal cuttings grow into microplants. These microplants may undergo another cycle of nodal cutting to increase the plant stock, or they may be grown on to produce full-sized plants.

The apparatus of the present invention has been used to produce potato plants for seed stock production. On average, using a manual system, 2,500 nodal cuts per day per person are produced. Using the apparatus of the present invention 150,000 cuts per day were produced.

The apparatus and process or method described for use in relation to potato cultures is also of course suitable for use with any plant species or variety to which axillary bud culture methods may be applied. The invention is particularly suitable for certain forestry species and plants such as tobacco, rhododendrons, chrysanthemums, top fruits and soft fruits, in addition to potatoes.

Furthermore, the invention may be applied to the subculture of plants which use adventitious regeneration as their pathway of propagation, in cases where the growth habit in vitro is conducive to being handled in this way, for example Sansereria gladioli.

A variant in the apparatus of the invention is shown in Figure 9. In this arrangement, apparatus according to the invention comprises an enclosure 101 in which cutting takes place. The enclosure 101 may be adapted for substantially contamination-free cutting, such as by the provision of a laminar-flow cabinet, or by sterilisation, using other means, of the internal environment within the enclosure. Such a feature or requirement is not however essential to the invention. The enclosure is provided with an arrangement, not shown in this diagrammatic pictorial representation, for holding an elongate tray 102 containing a multiplicity of microplants 103, in an inverted disposition. A suitable such arrangement may be provided by using laterally grooved trays in conjunction with a support feature such as the longitudinal ribs or ridges employed in the embodiment of apparatus described with respect to Figures 3 through 8. A cutting head 104, provided with a rotary circular blade 105, is mounted for travelling displacement in a direction extending along the length of the elongate tray 102. Cutting head traverse takes place along two guide members, 106-107, suitably in a sliding manner. The rotary blade 105 is mounted on the cutting head 104, for rotation in a plane 108 (Figure 11), extending substantially parallel to a plane defined by the growth tips 109 of the microplants 103. The rotary blade 105 is driven in rotation at high speed by a drive motor accommodated in the cutting head or carriage 104.

In the arrangement shown, blade drive and cutter head travel are independent. In order to effect a cutting operation, the rotary blade 105 is set in motion, the relative placement of the growth tips 109 of the microplants 103 is adjusted relative to the cutting plane 108 of the blade 105 so that the growth apices of the plantlets will

be cut off in the cutting operation, and the rotary blade 105 is then traversed along the length of the inverted tray 102, such as by manual displacement of carriage or cutting head 104, thereby severing the apices or nodes. The nodal cuttings fall into a collection region at the base of the enclosure 101.

Preferably, carriage drive is also motorized, as well as blade drive. In an adaptation of the structure shown, cutting head traverse may take place by at least one of the guide shafts 106 and 107 on which the cutting head moves being also drivable in rotation, for drive by an external motor mounted outside enclosure 101. This rotatable shaft 106 or 107 may serve as a lead-screw or like mechanism to effect cutting head traverse in both directions, with appropriate features for reversal of drive at the end of the traversing motion. The second rotatable guide shaft 107 or 106 may also be powered to drive the blade 105 in rotation through a suitable gear train within the cutting head 104. In an alternative construction, both drive features may be activated by a single rotatably mounted shaft, 106 or 107, again driven from outside the enclosure 101.

In this alternative cutter structure, the cutting takes place by virtue of the rotary action of the circular blade 105, as distinct from the scissors-type cut of the interdigitated blades previously described. The rotary blade thus provides a single-element cutter as herein defined. The rapidly moving cutting edge 111 of this blade 105 cuts through the plant material as the blade edge passes at great speed across the plant stem 112. In order to stabilise however the plantlet or "hedge" structure or array provided in the inverted tray 102, the apparatus of the invention preferably also comprises a plant-supporting structure, in the basic form of a pair of laterally displaceable bar members 113, suitably supported from the roof 114 of the enclosure 101 by arms 115 pivotally mounted on and depending from said roof 114. Bars 113 are located to the sides of a container or tray 112 when mounted in an inverted disposition in enclosure 101,

and are pivotable inwardly and outwardly relative to the plant array. Members 113 are moved inwardly therefore from the sides against the hedge or plantlet array to define and constrain the side edges of the array during a cutting operation. A suitable locking mechanism associated with the pivoting arms 115, such as an over-centre mechanism of known kind, is provided to retain the bar members 113 in their constraining or inwardly displaced disposition. Similarly, when the bar members are pivoted into their outward dispositions, the locking or over-centre mechanism associated with the pivotal mounting of arms 115 holds the bar members 113 in this clear or inactive configuration, in which placement and removal of microplant trays or containers 102 is facilitated. The lateral members 113 may also be provided with a series of inwardly directed teeth 116, Figures 10 and 11, which engage the plantlets 103 from the side of the container or tray 102. The teeth 116 approach one another towards the centre of the hedge or plant growth body, as shown in Figure 11, but do not meet or interengage or overlap in a meshing manner in the construction shown. Figure 11 shows the bar members 113 at the lower ends of the pivotal arms 115 in a co-operating plantlet constraining or hedge-defining disposition, in which the arms 115 have been pivoted inwards so that the bar members 113 engage gently against the sides of the outermost microplants 103 in the array of plantlets held in the tray or container 102. The teeth 116 penetrate between microplants of the array, and the inward ends of the teeth 116 of each bar member 113 are closely juxtaposed at the centre of the array, but do not meet, in the construction shown. Thus a series of generally rectangular zones are defined within the hedge or array, each zone having a boundary defined by portions of bar members 113 and teeth 116. This array of zones provides a multiplicity of bounded regions which constrain and support the plant array for a cutting operation. In this manner therefore, the interleaving structure of the teeth 116 and the sidewise support of the bar or lateral members 113 ensure a high quality of cut.

The high speed of rotation of the blade 105 is believed to establish a boundary layer in front of the blade, which may in certain circumstances tend to displace plants away from the blade during blade traverse in a cutting operation. The supporting structure provided as described above overcomes however any tendency of plantlets to move away from the advancing and rotating blade. Alternatively, the rotary blade 105 may be adapted on its lower face to establish a low pressure region beneath the blade, thereby counteracting any boundary layer action ahead of the blade tending to push the plant stem away by a counterbalancing pulling force beneath the blade.

The invention is especially effective in achieving sterile cutting. The high speed of rotation of the blade ensures that there is no significant accumulation of plant material on either surface of the blade. Alternative single-element cutters may however also be used, in particular non-physical cutters such as laser cutters.

As described in Figure 9, a single rotary blade 105 only is used. Figures 12 shows in diagrammatic plan view an alternative construction in which a plurality of contra-rotating planar circular blades 125a-f are used. Every second blade 125 a,c,e and 125 b,d,f rotates in the opposite direction and the structure is arranged to have an even number of blades, so that the outer blades on each side 125a and 125f rotate inwardly, and thereby tend to accentuate and contribute to the holding action of the bar members 113, where present, or to gather the hedge inwards, in the case of an unconstrained plant array.

The invention may also be applied to upright cutting of microplants, in the manner shown in Figure 13. In this instance, a sheath or cover 131 comprising a substantially planar member is placed above the rotary blade 105. Cut plant nodes 132 falling away from the hedge or plant array 103 are collected on this sheathing surface 131, and may be swept away by suction or any other relevant suitable

arrangement. A multiplicity of further possibilities then exist in regard to further handling of cut plant nodes, following collection after severance, for subsequent downstream placement for further growth and development, including air streaming or entrainment in carrying and conveying media of other kinds.

CLAIMS

1. Apparatus for severing plant material in micropropagation comprising:

(a) cutting means, and

(b) means for holding a multiplicity of microplants for a cutting operation, wherein the disposition of the holding means during a cutting operation is such that plant material severed from said multiplicity of microplants during said cutting operation falls free of said multiplicity of microplants.

2. Apparatus for mechanisation of plant micropropagation, comprising a chamber maintainable under sterile conditions and housing a holding means for a culture container containing microplants and a cutting means for cutting the microplants into nodal cuttings, the holding means being arranged to hold the container in a position such that the nodal cuttings fall free of the microplants and container, once cut.

3. Apparatus according to Claim 1 or Claim 2, wherein the holding means is arranged to hold a culture container containing microplants in an inverted disposition with respect to the normal growing position so that the microplants are suspended from the culture container above the cutting means.

4. Apparatus according to Claim 3, comprising means for raising or lowering the culture container with respect to the cutting means, so that successive cuts may be taken from the microplants.

5. Apparatus according to Claim 3, comprising means for adjusting the relative disposition of the culture container relative to the cutting means so that successive cuts may be taken from the microplants.

6. Apparatus according to any of Claims 3 to 5, comprising means for aseptically sealing a container holding newly-produced micro-cultures obtained by cutting.

7. Apparatus according to Claim 6, wherein the sealing means comprises a mechanism for holding one or more rolls of plastics film for feeding around a culture container to be sealed.

8. Apparatus according to any preceding claim, wherein said cutting means comprises at least one single-element cutter.

9. A method of severing plant material in micropropagation comprising holding a multiplicity of microplants for a cutting operation in a disposition such that plant material severed from said multiplicity of microplants during said cutting operation falls free of said multiplicity of microplants.

10. A mechanised process for micropropagation of plants in which microplants are held in a sterile environment and are inverted over a mechanical cutting means for cutting nodes from the microplants for collection on a culture-medium-containing tray located beneath the cutting means.

11. A process according to Claim 10, wherein a tray containing cut nodes is aseptically sealed and cultured to produce microplants.

12. A method of micropropagation including the steps of:

presenting at a workstation a plurality of plantlets held in a nutrient medium, the medium being presented in an orientation such that a portion of a plantlet which is cut from the plantlet falls free from the plantlets held in the nutrient medium,

cutting through a plurality of the growing plantlets to release in bulk cut-away plant portions large enough to contain tips and/or nodes, and

receiving the released plant portions on a second nutrient medium.

13. Apparatus for use in micropropagation of plants comprising

a first support means for supporting a nutrient medium with plantlets growing in the medium,

means for presenting the nutrient medium and plantlets in an orientation such that a portion of a plantlet that is cut from the plantlet falls free of the plantlets held in the nutrient medium,

cutting means for cutting through a plurality of growing plantlets to release cut-away plant portions large enough to contain tips and/or nodes, and

second support means for supporting a second nutrient medium, the second support means being arranged in a position such as to receive in bulk the released plant portions cut by the cutting means.

14. Apparatus for severing plant material in micropropagation comprising:

(a) a cutting head having at least one single-element cutter,

(b) means for holding a multiplicity of microplants for a cutting operation,

(c) means for effecting displacement of said cutting head relative to said microplants, and

(d) means for establishing a cutting relationship between said single-element cutter and said microplants.

15. Apparatus according to Claim 14, wherein said single-element cutter comprises a circular blade drivable in rotation.

16. Apparatus according to Claim 15, wherein said means for establishing a cutting relationship comprises drive means for said circular blade.

17. Apparatus according to any of Claims 14 to 16, wherein said cutting head is mounted on a carriage for said displacement of said cutting head relative to said microplants.

18. Apparatus according to Claim 17, wherein said carriage is mounted on guide members for travelling displacement of the carriage and at least one of said guide members is drivable in rotation to effect said travelling displacement of the carriage.

19. Apparatus according to Claim 18, wherein at least one of said guide members is drivable in rotation to establish said cutting relationship between said single-element cutter and said microplants.

20. Apparatus according to any of Claims 14 to 19, comprising means for constraining the microplants against significant displacement in at least one direction during a cutting operation.

21. Apparatus according to any of Claims 14 to 20, wherein the disposition of the holding means during a cutting operation is such that plant material severed from said multiplicity of microplants during said cutting operation falls free of said multiplicity of microplants.

22. Apparatus for severing plant material in micropropagation substantially as described herein with reference to and as shown in any one of more of the accompanying drawings.

23. A method of severing plant material in micropropagation substantially as described herein with reference to any one or more of the accompanying drawings.

24. Microplants prepared by the method or process of any of Claims 9 to 12 or 23.

25. Microtubers prepared by the method or process of any of Claims 9 to 12 or Claim 23.

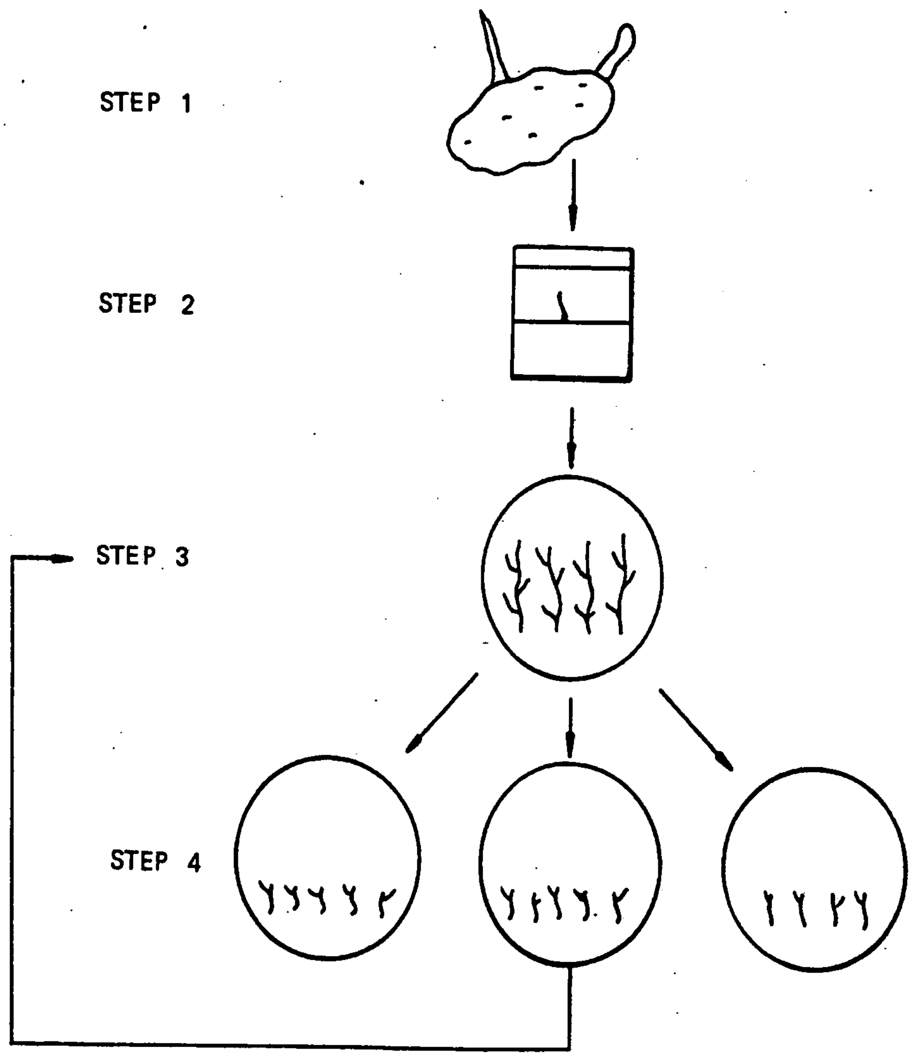

FIG. 1

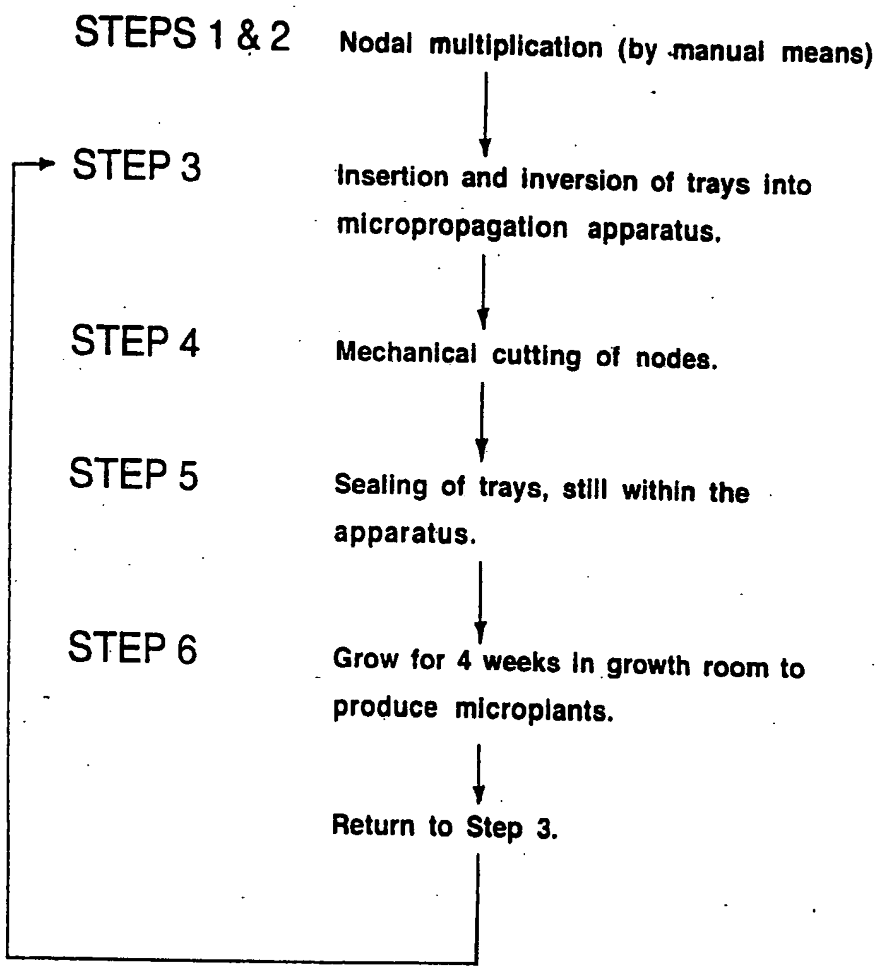
STEPS 1 & 2
Nodal multiplication (by manual means)
STEP 3
Insertion and inversion of trays into micropropagation apparatus.
STEP 4
Mechanical cutting of nodes.
STEP 5
Sealing of trays, still within the apparatus.
STEP 6
Grow for 4 weeks in growth room to produce microplants.
Return to Step 3.

FIG. 2

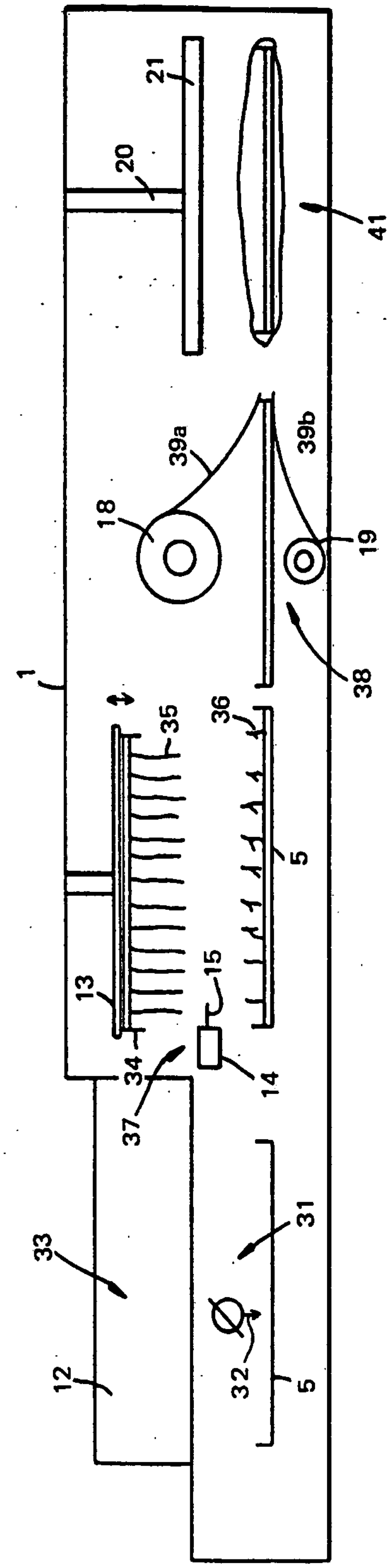

FIG. 3

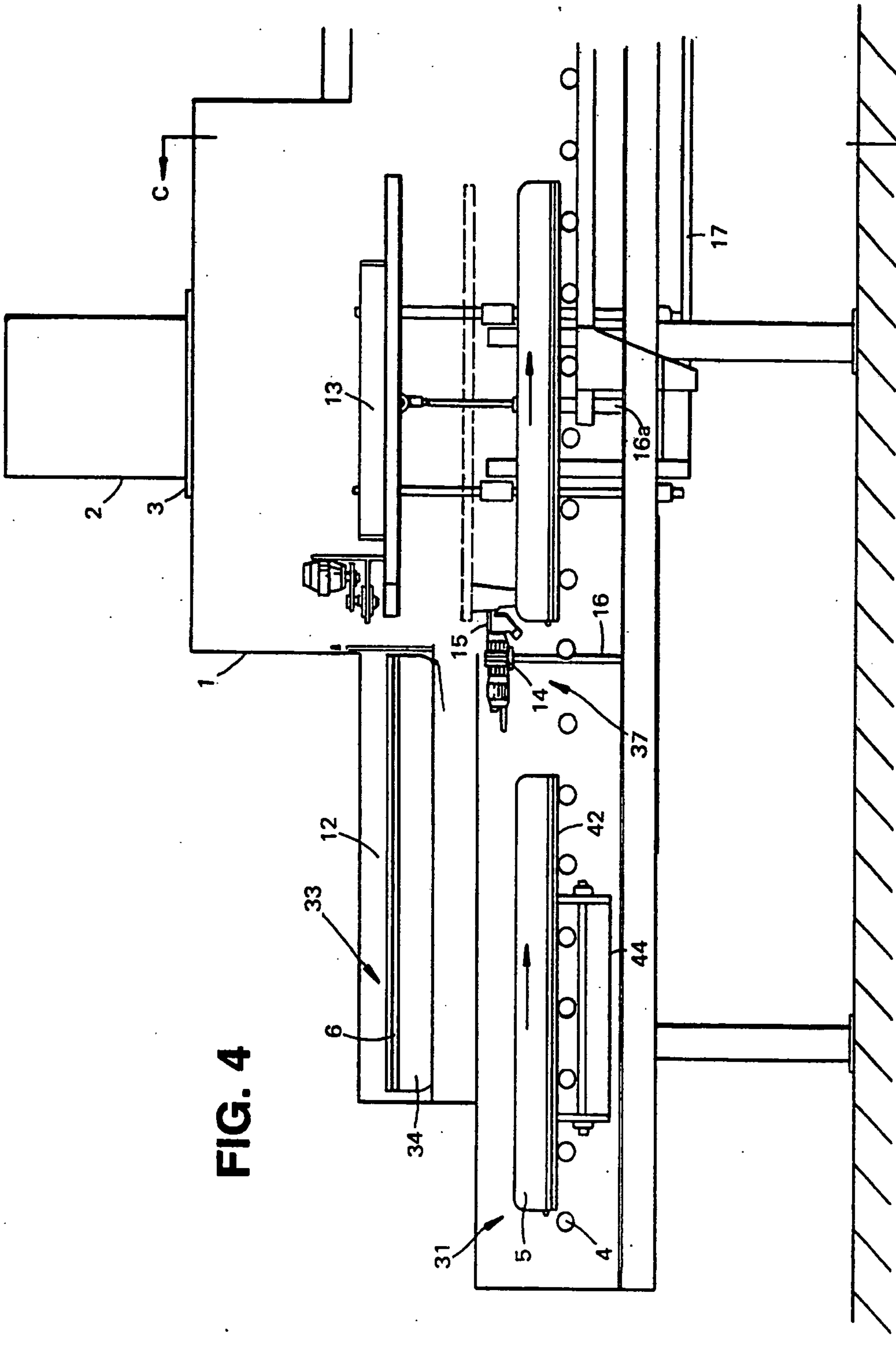

FIG. 4

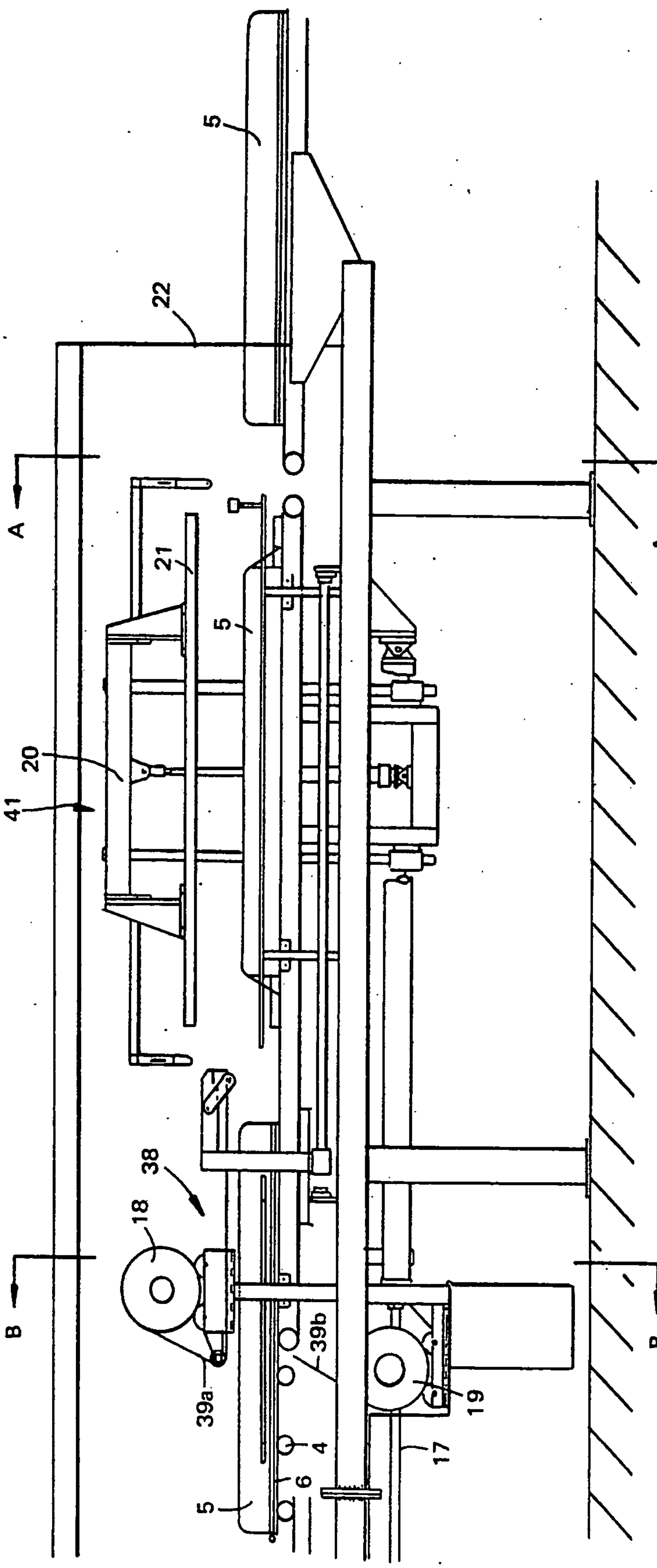

FIG. 5

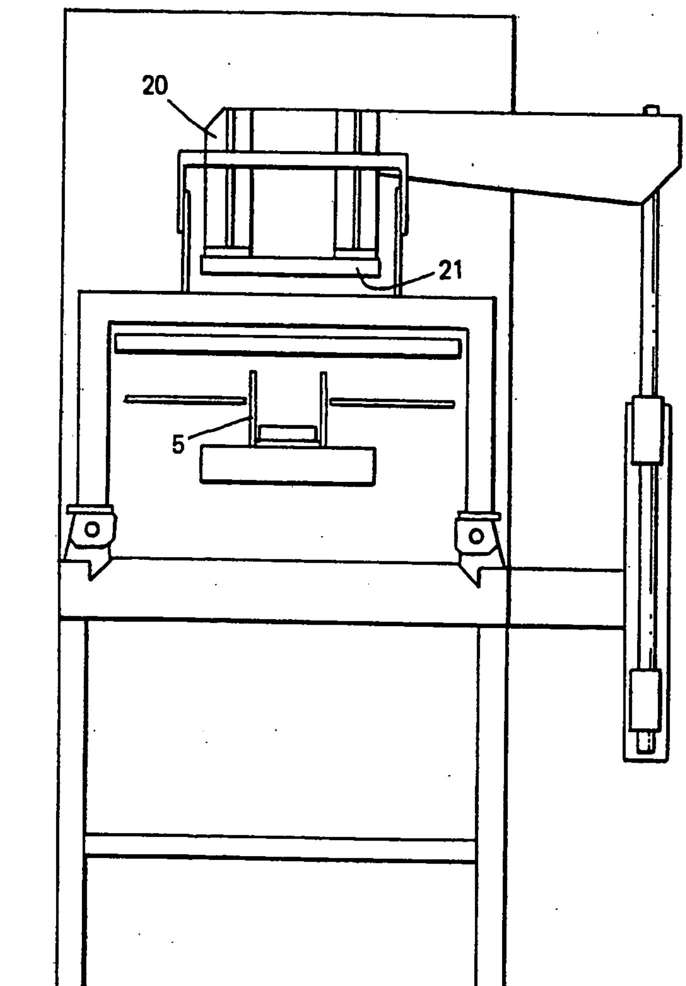

FIG. 6

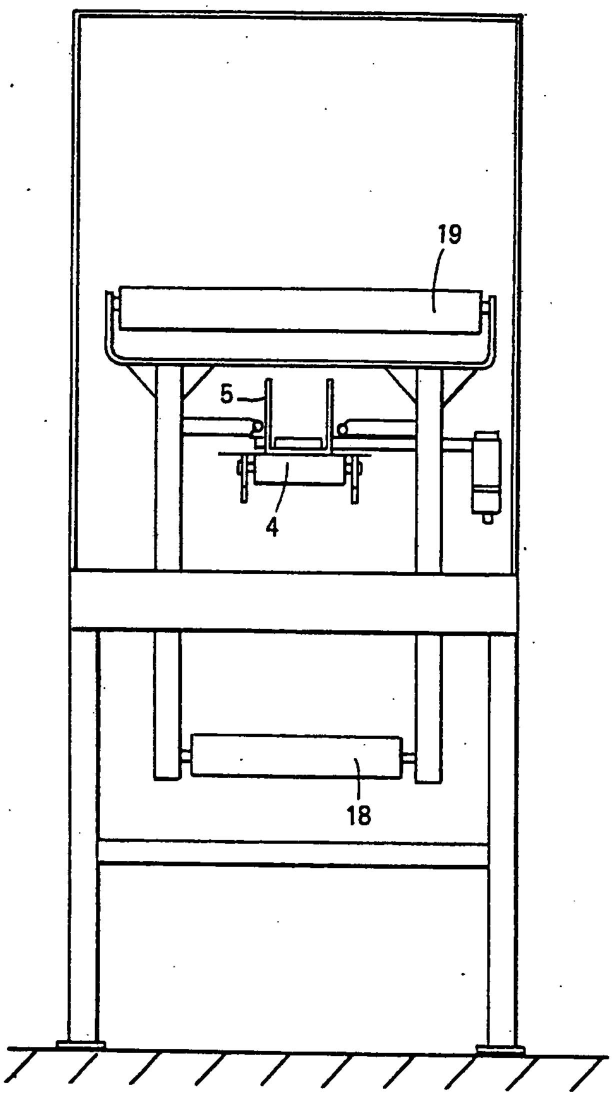

FIG. 7

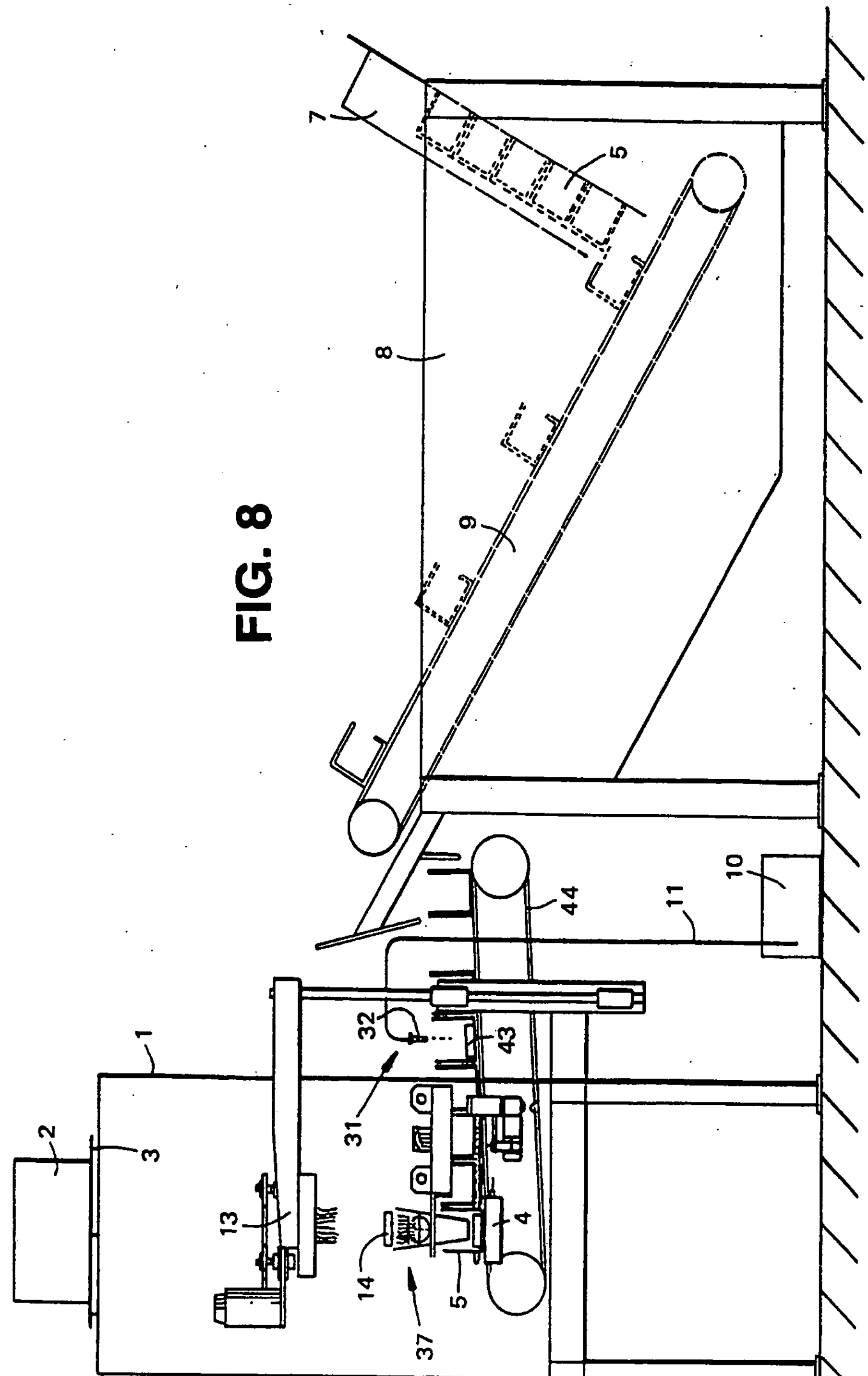

FIG. 8

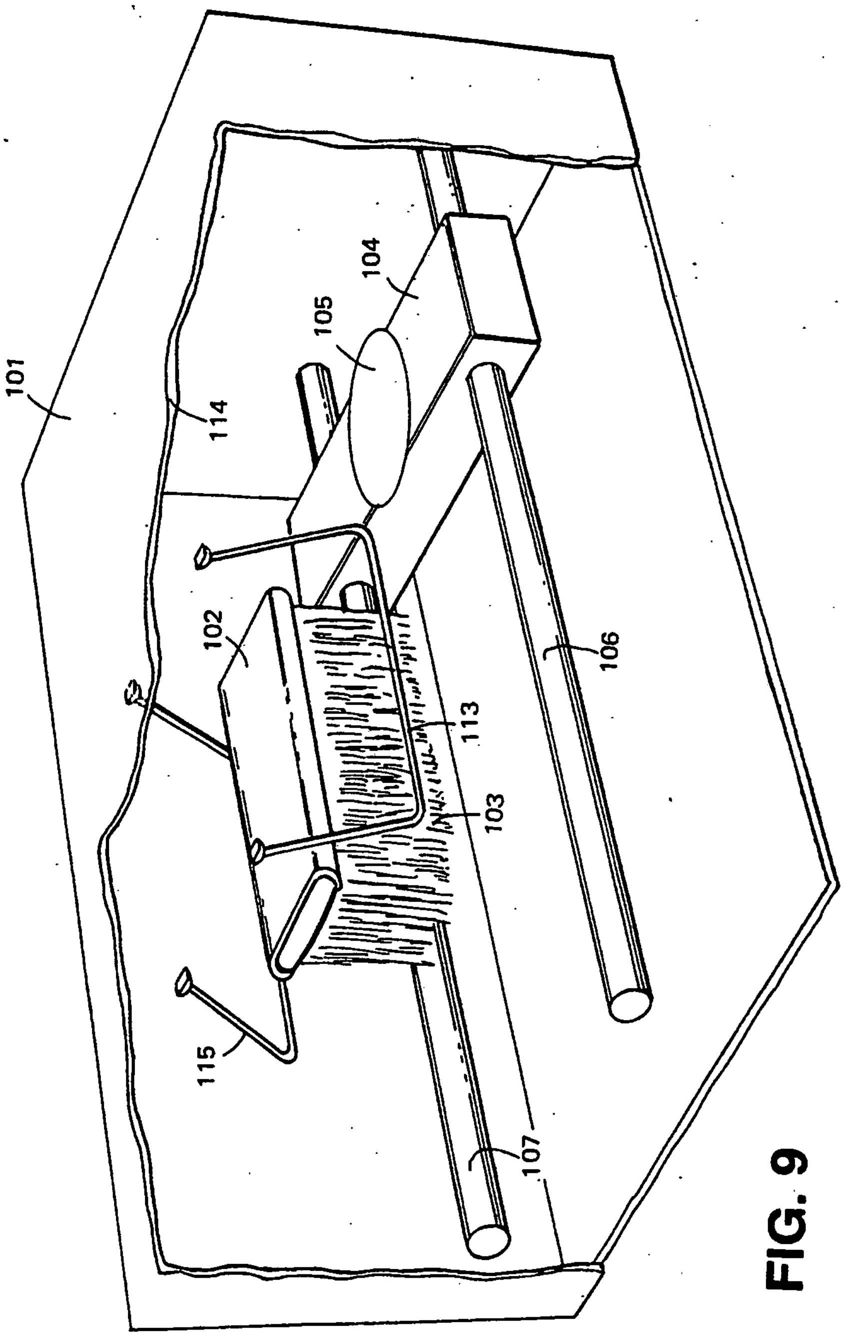

FIG. 9

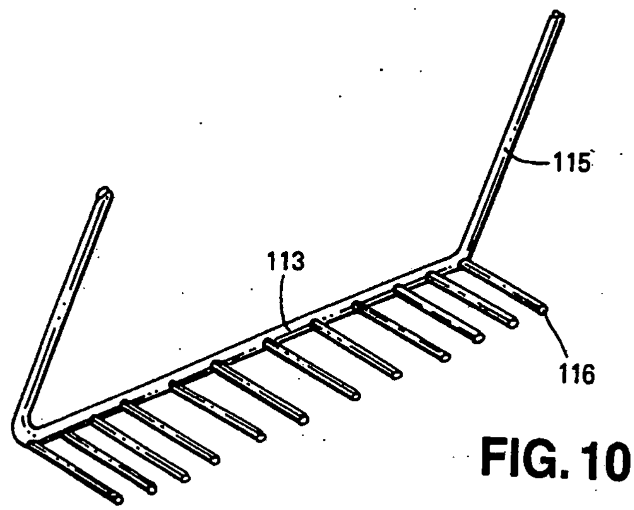

FIG. 10

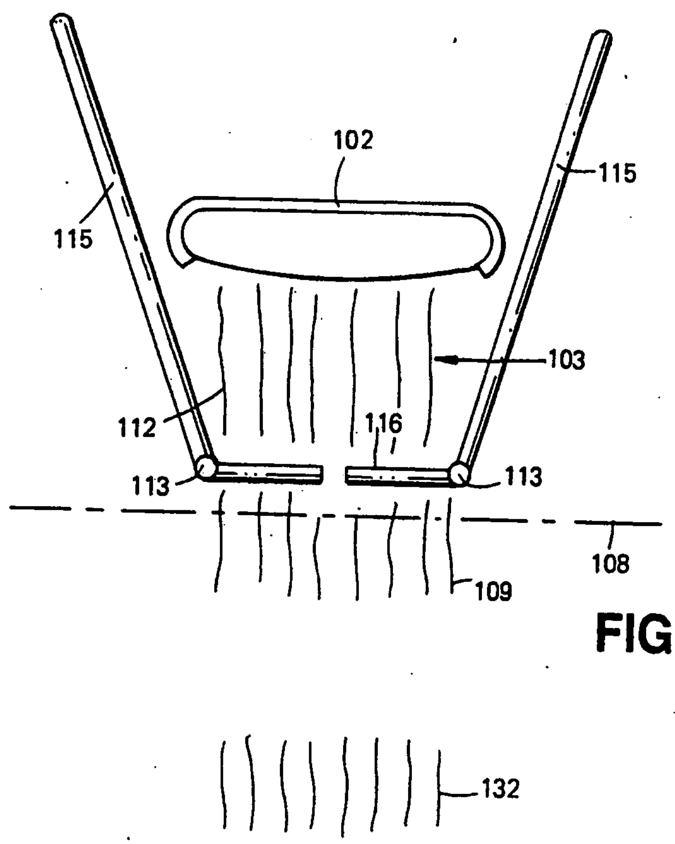

FIG. 11

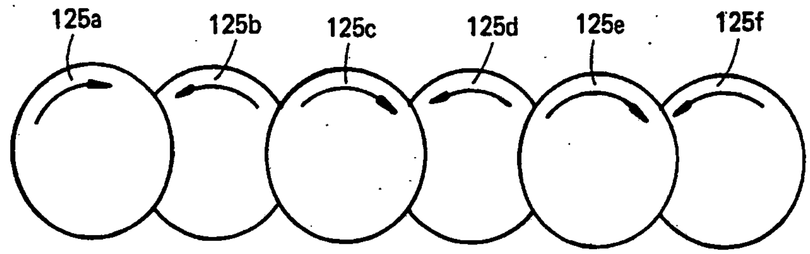

FIG. 12

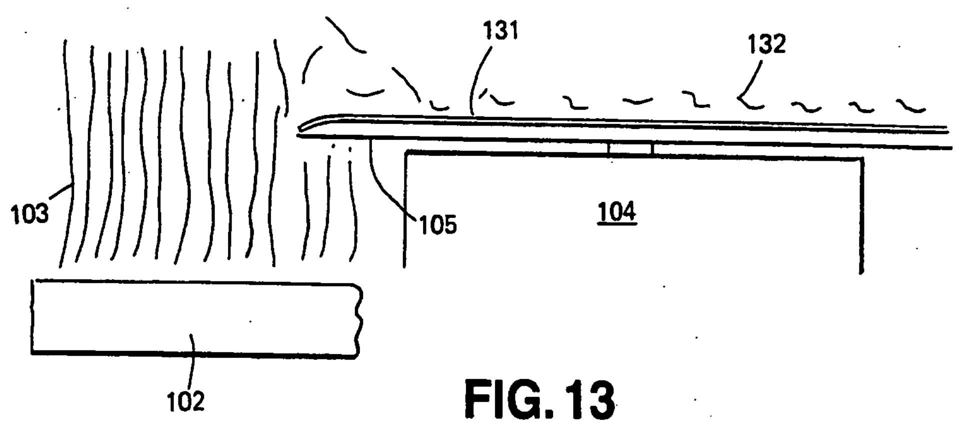

FIG. 13

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/00703

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 A01H4/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A01H

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,9 115 110 (ALLARD) 17 October 1991 see page 10, line 13 - page 15, line 25; figures 1-4 ---	1-5, 8-10, 12-14, 20-25
X A	WO,A,9 103 929 (NATIONAL RESEARCH AND DEVELOPMENT CORPORATION) 4 April 1991 see page 11, line 23 - page 12, line 14; see page 25, line 18 - page 27, line 4; figures 1,4,11,13,14 ---	1-5, 8-10, 12-15 16
A	EP,A,0 389 019 (PLANT PRODUCTION SYSTEMS BV) 26 September 1990 see column 3, line 50 - column 5, line 47; figure 1 --- -/--	1,2,6,11

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

3

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
12 OCTOBER 1992	20. 10. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	DISSEN H.D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,8 806 618 (KONTAKTA ALKATRESZGYAR SZENTESI GYARA) 7 September 1988 see page 5, line 10 - page 8, line 12; figure 1	6,7,11
A	EP,A,0 449 366 (PERMX BV) 2 October 1991	
A	WO,A,9 006 058 (AGRISTAR INC) 14 June 1990	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9200703 SA 58467

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9115110	17-10-91	AU-A- 5839890	30-10-91
WO-A-9103929	04-04-91	AU-A- 6400990	18-04-91
		EP-A- 0493429	08-07-92
		GB-A- 2236069	27-03-91
EP-A-0389019	26-09-90	NL-A- 8900555	01-10-90
		AU-A- 5260190	09-10-90
		EP-A- 0462173	27-12-91
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		GB-A- 2209535	17-05-89
		NL-T- 8720126	01-02-89
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		WO-A- 9114360	03-10-91
WO-A-9006058	14-06-90	US-A- 4978505	18-12-90
		AU-A- 4812990	26-06-90
		US-A- 5088231	18-02-92

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82